HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta – Fakultet – Faculty Faculty of Biological and Environmental Sciences	Koulutusohjelma – Utbildingsprogram – Degree Programme Ecology and Evolutionary Biology	
Tekijä – Författare – Author Brittni Joette Crosier		
Työn nimi – Arbetets titel – Title Patterns of Distance Decay in Finnish Fungal Communities		
Oppiaine/Opintosuunta – Läroämne/Studieinriktning – Subject/Study track Ecology		
Työn laji – Arbetets art – Level Master's thesis	Aika – Datum – Month and year May 2020	Sivumäärä – Sidoantal – Number of pages 25 + appendices

Tiivistelmä – Referat – Abstract

Biogeography is a crucial aspect to ecological studies, as an ecosystem is comprised of the physical habitat, the organisms living there, and the interactions of these components. Community structure, and therefore functioning, are inherently of a spatial nature. Spatial structure of populations is often crucial basic knowledge for understanding the evolutionary history, dispersal patterns, and resilience of any given species. One aspect of spatial structure, and the topic covered in this study, is community distance decay, or the rate at which community similarity decreases with physical distance.

More of the landscape is constantly being altered by humans on a large scale, so it is increasingly important to understand the effects that these anthroprogenic changes to the environment has on local populations. Studying community distance decay helps form understanding of dispersal and establishment limitations for different organisms, which is necessary for mitigating biodiversity loss. Many studies show that habitat fragmentation and loss has greatly impacted the structure of plant and animal communities, but there has been much less focus on fungal communities. There's no certainty that fungi is impacted in the same ways, given the different lifestyles and dispersal methods, so the aim of this study is to contribute to the much needed research on fungal community structure at various scales.

This aim is addressed by examining fungal community distance decay from small scale of a couple kilometers or less to a fairly large scale encompassing a landscape of primarily urban, forest, and agricultural areas. The five main localities of sampling were in middle and southern Finland: Helsinki, Lahti, Tampere, Jyväskylä, and Joensuu. Sampling locations and plot design were chosen to allow the comparison of communities separated by a mosaic, as well as along a short rural to urban gradient, to assess the effects of habitat type. From each location, six plots were decided, two in urban core, one in urban edge, two in natural core, and one in natural edge.

The role of dispersal ability and functional traits in distance decay is also studied by comparing results from two different methods of fungi sampling, which were collecting spores from the air using cyclone samplers, and taking soil cores to gather fungal biomass. All samples were DNA analysed with high-throughput sequencing and the results from the DNA barcoding were used to create OTU clusters, by which the 30 plots could be compared through relative abundances of OTU's.

I determined the similarity of fungal communities using an analysis of similarity (ANOSIM) test in R, where all possible variables (site, habitat type, sample type) were used as a grouping in individual tests, thereby indicating which variable is associated with highest community difference. I also determined the differences in functional groups and major taxonomic levels among locations and sampling method using interactive taxanomic (KRONA) charts.

Results showed that there are differences in fungal community structure among habitat type and sampling type. However there was greater difference at the level of plots than site locations, so clear patterns of strong community distance decay with physical distance was not measured in this study.

The results suggest that fungal communities can be fairly impacted by human caused habitat change, and individual characteristics, such as dispersal methods or lifestyle, effect the rate of community distance-decay. This provides a valuable early insight into fungal community patterns, which need deeper study to understand the complexities and mechanisms behind them.

Avainsanat – Nyckelord – Keywords
community simmilarity, dispersal, diversity, spores, soil

Ohjaaja tai ohjaajat – Handledare – Supervisor or supervisors
Otso Ovaskainen

Säilytyspaikka – Förvaringställe – Where deposited
E-Thesis

Muita tietoja – Övriga uppgifter – Additional information

Patterns of Distance-Decay in Finnish Fungal Communities

By: Brittni Joette Crosier

Master's Thesis

Faculty of Biological and Environmental Sciences
University of Helsinki
Master's Degree in the Ecology and Evolutionary Biology Programme

May 2020

Table of Contents

Introduction 4

1. Causes and patterns of spatial structure in ecological communities.....4
2. Known patterns of distance decay 4

2.1 Other taxa.....4

2.2 Fungal and microbial 5

3. How fungal dispersal patterns effects community structure.....5

3.1 Spore dispersal patterns.....5

3.2 Fungal soil patterns.....6

4. Effects of urban vs. natural habitats on ecological communities.....6

4.1 Biotic factors.....6

4.2 Abiotic factors.....7

5. The purpose of this study.....7

5.1 Aims.....7

5.2 Hypotheses.....8

Materials and methods 8

6. Field sampling.....8

6.1 Site selection & plot design.....8

6.2 Spore sampling methods.....9

6.3 Soil sampling methods.....12

7. DNA Extraction & Sequencing.....12

7.1 Sample preparation.....12

7.2 DNA pipeline.....12

7.3 Clustering and species identification.....12

8. Statistical analysis.....14

Results.....14

9. Qualitative observations.....14

9.1 Patterns by location.....14

9.2 Patterns by Sampling type.....14

10. Quantitative analyses.....16

Discussion.....16

Acknowledgements.....20

References.....21

Appendices.....26

Introduction

1. Causes and patterns of spatial structure in ecological communities

Understanding the spatial structure of communities is an essential component for the study of biological processes, ecosystem functioning, and evolution. Spatial ecology examines the pattern dynamics of biological communities and environmental factors at different spatial scales, such as regional, global, or local landscapes (Meentemeyer et al, 1987; Wiens 1989). At a level of landscape ecology, patterns are typically considered within a mosaic of habitat patches (Picket &Cadenasso, 1995; Xiao et al., 1997). There is increasing interest in how mosaic structure can influence plant, animal, and fungi population distribution, and simultaneously how biological processes like dispersal and establishment create patch dynamics (Dale & Fortin, 2014).

Distance-decay, in ecology, is the decreasing similarity in communities with increasing physical distance (Nekola & White, 1999; Soininen & Hillebrand, 2007; Morlon et al., 2008). It is one important aspect of biogeography, which should aim to encompass both patch characteristics and individual organism attributes (Wiens et al., 1993). Distance-decay rates will be greatly affected by the nature of the matrix, with things like patch size and connectedness determining an organisms' ability to move between and persist within habitat patches (Nekola & White, 1999).

2. Known patterns of distance decay

Two main categories of cause for distance-decay have been identified and studied: difference in environmental similarity, such as a latitudinal or climatic gradient (Qian & Ricklefs, 2007; Qian et al., 2009), and spatial configuration, relating more to size and composition of habitats within a landscape (Garcillán & Ezcurra, 2003). While the former can be addressed with long-term competition and evolution studies, this study will address the latter by studying how fungi are distributed across a habitat matrix with relatively similar environmental conditions.

2.1 Other taxa

There have been many studies examining the distance-decay patterns of specific taxa, functional groups, and species (Green et al., 2004; Novotny et al., 2007) finding that all can be impacted differently by landscape features.

Dispersal ability is known to be a major factor for distance decay in plant communities. One component is individual mechanisms, with spore plants and microseeded plants having much lower rates of distance decay than those relying on larger fruit (Nekola & White, 1999). Fungi, primarily dispersing through spores, would likely have distance-decay patterns resembling the spore plants and microseeded plants.

In addition to individual mechanisms, species distribution can be impacted by a wide range of external biotic and abiotic factors, such as environmental degradation and abundance of symbiotic species **(**Pérez-del-Olmo et al., 2009)**.** Habitat fragmentation is one external factor, which in combination with dispersal ability, impacts community similarity in many taxa like plants (Nekola & White, 1999; Chust et al., 2006), pollinator insects (Jauker et al., 2009), and fish (Kornis et al., 2015)

2.2 Fungal and microbial

In general, the distance decay of some microbial communities, including fungi, is similarly impacted by the same external factors as other taxa. The common main factors of distance decay, particularly changes of environmental similarity, hold true for the distribution of fungal communities, such as distance from the equator, which is one of the major factors in overall species richness (Taylor & Gaines, 1999), and applies also specifically to ectomycorrhizal fungi (Bahram et al., 2013; Tedersoo et al., 2014). Ectomycorrhizal fungi also rapidly changes in distribution and abundance along a forest productivity gradient (Kranabetter et al., 2009) and altitudinal gradients (Bahram et al., 2012). At a smaller scale, community composition of wood-decay fungi differs with physical distance and habitat factors such as dead-wood resources and forest management style (Abrego et al., 2014)

3. How fungal dispersal patterns effects community structure

3.1 Fungal spore dispersal

Fungi disperse through the air by means of spores, released from fruiting bodies called mushrooms. There is increasingly more interest and research into spore dispersal patterns, environmental limitations, and establishment success (Edman &Ericson, 2004; Vernes & Dunn, 2009; Peay & Bruns, 2014; Norros et al, 2015), but there are still large gaps in the knowledge. Limitations in small-scale spore dispersal and establishment mean some wood-decay fungi may

have difficulty colonizing fragmented habitats and habitat edges (Norros et al, 2012). Spore dispersal limitation also drives community dissimilarity in some Basidiomycete fungi (Peay & Bruns, 2014). Therefore, studying fungal spore similarity throughout mosaic landscapes may give insight on whether these patterns hold true for most fungal species across a hierarchical scale.

3.2 Fungal soil patterns

The study of spatial variability of soil communities is relatively recent, lagging behind other aspects of spatial ecology, but its importance should not be underestimated in the whole ecosystem biogeography. Of the studies which look at soil fungi variability with distance, most have focused on vertical, rather than horizontal, difference (Jumpponen et al, 2010; Santalahtiet al., 2016) However there are some studies that answer how fungal soil communities change over horizontal space, mostly focused specifically on mycorrhizal fungi (Bahram et al., 2013; McGuire et al., 2013; Bahram et al., 2014). Spatial variability is higher in the topsoils than at greater depths (Bahram et al., 2014), possibly demonstrating the importance of environmental heterogeneity in fungal community similarity.

4. Effects of urban vs. natural habitats on ecological communities

Urban environments differ in many fundamental ways from surrounding areas with less human impact. Many aspects of ecological function are impacted by urban land use (Alberti & Marzluff, 2004), and researchers have acknowledged the importance of studying urban settings as unique ecosystems (Niemelä, 1999; Breuste et al., 2008).

4.1 Biotic factors

Distinct biodiversity patterns exist in urban environments. Research on urban vegetation trends found that urban environments, particularly towards the core, have more exotic species and less native species (Rudnicky & McDonnell, 1989; Kowarik, 1990; Rapport, 1993). Many types of fungi rely on plants as hosts, and unsurprisingly, plant host density is much lower in urban areas compared to forested counterparts (Bahram et al., 2013).

Soil communities in urban areas differ from those in rural areas in some ways that impact fungal populations. Cities have slower litter decomposition (Carreiro et al., 1999) and lower total fungal biomass (McDonnell et al., 1997). Further differences in fungi can be expected from

interactions with other soil organisms. Earthworms, which have higher abundance in urban areas (Steinberg et al., 1997; Baxter et al., 1999), change fungal composition through mycophagy (Bonkowski et al., 2000) and their effects on litter decomposition and soil nitrogen (Willems et al., 1996). Nematodes, which vary in trophic function, have a lower fungivore to bacterivore ratio in urban soils (Pavao-Zuckerman & Coleman, 2007).

4.2 Abiotic factors

Urban patches generate their own microclimate, often with higher temperature, known as the Urban heat island effect (Oke, 1973), moisture, and pollution levels. (Taha, 1997). Heat and moisture have a large role on the success of air dispersal of fungi, and the same factors that trap pollution particles could cause more spores to be retained. Therefore, the microclimate of urban areas could likely lead to different spore assemblages. Furthermore, structural features such as buildings may serve as dispersal barriers.

Soil composition also changes with urban land use. Along an urban to rural gradient, there are higher concentrations of heavy metals and higher acidity towards the core (Pouyat et al., 1995). Urban soils differ in basic nutrients, too, with more phosphorous and higher rates of nitrogen mineralization (Pouyat et al., 1995; Wernicket al., 1998). These things likely result in different soil fungal communities.

5. The purpose of this study

5.1 Aims

The aims of this study are to 1) increase understanding of the individual spatial processes of fungi across many functional groups, both by studying community distance-decay at local and regional scales, and by comparing fungi found in soil to spores in the air 2) contribute to the still relatively few studies of impacts from urbanization and landscape fragmentation on fungal community structure. Despite the increasing body of literature on fungal biogeography, there are still large gaps in our knowledge about large-scale distribution of species and how they are impacted by environmental factors (Peay et al., 2010). Studies such as this, analyzing distribution over a regional scale and differentiating between habitat types, will help address parts of this question.

5.2 Hypotheses

H1: Populations of fungi in the study area will exhibit the pattern of decreasing community similarity with increasing distance, following broad trends of community distance decay in studied organisms.

H2: Functional groups of fungi will be differentially affected by ecosystem patch mosaics and fragmentation.

H3: Samples of soil fungi will have higher rates of distance decay than spore samples.

H4: Distance-decay will vary along an urban to rural gradient, with rates increasing with proximity to urban cores.

Materials and Methods

6. Field Sampling

6.1 Site Selection & Plot Design

Sampling was conducted in five locations across southern Finland: Helsinki, Lahti, Tampere, Jyväskylä, and Joensuu. The locations were selected to create a network of sampling which allows comparison between many different distances, ranging between 70 kilometers apart at the nearest locations and 350 kilometers at the furthest locations. This range was chosen in order to cover large enough distances to see the effect of distance-decay on fungal communities, while also keeping sites close enough, particularly in terms of latitude, to be in similar climatic regions. All study locations are within three degrees of latitude to minimize effects of climatic gradient, so observed community differences are more likely due primarily to dispersal limitations or patch effects.

Each location includes an urban site and another site without much urban development, hereafter referred to as the natural site. In this study, urban areas are determined by population, and the five locations are within the fifteen largest cities in Finland (World Population Review, 2019). The natural sites are forested areas neighboring the urban counterpart.

Table 1: Population data comes from Official Statistics of Finland (OSF): Population structure [e-publication].

	TOTAL POP. (31.12.2018)	POP. DENSITY (PEOPLE/KM2)	LATITUDE
HELSINKI	648 042	2,934	60.17° N
LAHTI	119 951	262.35	60.98° N
TAMPERE	235 239	448.76	61.50° N
JYVÄSKYLÄ	141 305	120.73	62.24° N
JOENSUU	76 551	32.14	62.60° N

Samples were collected at three plots in each site. The plots were selected so that two are very close to each other, ca 1-2 km apart in the core of the site, and one is ca 10 km from the others, on the edge of the habitat type. The assessment of core and edge was made based on geographic distance to the city center or where habitat type began to change from city to suburban or from suburban to forest. This design allows for more fine-scale analysis of community changes that might happen at much smaller distances than between the five major locations. Furthermore, this creates an urban to rural gradient to observe impacts of urbanization on fungal communities.

At all thirty plots, four replicates were taken of both soil and air samples, except for four because of technical problems, resulting in 116 air samples and 120 soil samples. Of these, ninety of each sample type were chosen to be DNA analyzed, capturing within-week comparisons and between-week comparisons.

All samples were collected at the end of August, during a time with a relatively high volume of mushroom fruiting bodies to maximize spore quantity.

6.2 Spore Sampling

Spore samples were collected using cyclone samplers (Burkard Cyclone Sampler for Field Operation, Burkard Manufacturing Co Ltd; Emberlin & Baboonian, 1995), which actively collect airborne particles, including fungal spores, from the surrounding air and deposits the contents into an Eppendorf tube. Early studies by the Global Spore Sampling Project have been successful in using this method to assess fungal distribution and dispersal (Abrego et al., 2018; Ovaskainen et al., 2020).

Samplers were placed on the ground, in areas with enough space around for sufficient airflow, and sterile Eppendorf tubes in the collection chamber were used to contain the sample. Sampling was conducted in 24-hour periods for two consecutive days and a total of eight days in each location, alternating between natural and urban sites. On day one, three samplers were set up at each urban site and operated for two days. At the end of the sampling day, 24 +/- 1 hours, the sample tube was collected, closed, labeled with a unique ID, and replaced. After two samples were collected from each, they were switched to the natural counterparts to repeat the process for two 24-hour samples. The cyclone chamber was cleaned between sites to avoid contamination. After the first round of samples were collected from every site, the sampler batteries were recharged for one day, before conducting a second round of sampling to get replicates from each site, following the same design. The spore samples were kept cool until lab processing, and then stored in –20 C freezers, until sending them for DNA analysis.

Figure 1: Photographs of the Burkard Cyclone Samplers running in the field during sample collection. On the left is a natural forest plot near Helsinki (N2), and on the right is a plot in downtown Helsinki (U2).

6.3 Soil Sampling

Each time spore samples were collected, soil samples were taken in the same plots, within ten meters of the cyclone sampler. Soil was taken from patches that seem to be representative of the immediate area. The additional replicates per 10-meter plot can capture more variation in soils, which may only be spatially autocorrelated up to two meters in in boreal and temperate ecosystems (Häkkinen et al., 2011).

First litter was removed from the soil surface, and then soil cores were collected with a 2.5 cm diameter cylinder, to 5cm depth (or until reaching rock). Three subsamples were collected within a 1-meter square for each sample. The three subsamples were homogenized and pooled in the field. They were kept in cool storage until processing in the lab.

7. DNA Extraction & Sequencing

7.1 Sample preparation

First samples were processed in the lab. For soil, two milliliters were taken from the composite sample and added to a final sample tube and freeze-dried for 48 hours at 0.57mbar vacuum and -80°C temperature. These dried samples were stored in –20° C freezers until being shipped for DNA analysis.

Spore sample tubes were cleaned of larger items (eg: arthropods). First, sterile water was added to the sample tube and then the sample was put on Vortex to ensure that spores were released and not removed with the larger items. Then the large items were taken from the tube using sterile tweezers. After being cleaned, spore samples were covered with parafilm and freeze-dried for 24 hours at 0.57mbar vacuum and -80°C temperature. Dry samples were stored in –20°C freezers until being shipped for DNA analysis.

7.2 DNA pipeline

Samples were sent to the University of Guelph in Ontario, Canada, where extraction and analysis was carried out on the ITS region of fungal DNA, following the protocol for soil from Ivanova et al (2008) and the protocol for air used in the Global Spore Sampling Project (Ovaskainen et al, 2020). More detail can be found in Appendix B.

7.3 Clustering and species identification

OTU sequences were identified to taxonomic classifications with the statistical tool PROTAX-fungi, or PRObabalistic TAXonomic placement (Abarenkov et al., 2018), which uses the most up-to-date taxonomic classification system (IndexFungorum) and a reliable reference sequence database, UNITE (Somervuo et al., 2017), to place OTUs in a taxonomic group. Taxonomic classifications are also given a probability of correct identification, accounting for missing reference sequences or unknown species, and only sequences which could be reliably (>90%) classified into a known fungal phylum were included in analyses.

For the purpose of qualitative analyses, KRONA wheels (Ondov, et al., 2011) were constructed with seven-levels of taxonomy, showing percentages of taxonomic groups within the overall composition (Fig. 2). Taxonomic classifications are ranked on a confidence scale and assigned a color corresponding to the ranking. These graphs were created for each location and separated by soil and air samples, for ease of qualitative comparisons.

Figure 2: Above is a snapshot of the interactive KRONA charts, in which the order Agaricales has been selected and lower levels of taxonomy and their percentage of representation in the DNA is shown.

8. Statistical Analysis

Of the 180 samples, six were excluded from analyses because of sequence failure, resulting in less than 10,000 sequences in each of those samples. With the remaining, two matrices were created, one containing total DNA amount per sample, and one containing relative abundances, so that sums of each row were one. Comparisons between sites, habitat types, and sample types were made based on relative abundance, or proportion of total fungal sequences, rather than raw number of OTUs.

To measure the differences in community composition, the analysis of similarity (ANOSIM) test was used from the R package "vegan." The test was run multiple times, with all parameters staying the same, except for the grouping, which changed to address each variable being considered as a possible cause of community dissimilarity. As air and soil samples were not directly comparable, data was first separated into two tables based on sample type, and then each test was run individually for each type.

Results

9. Qualitative observations

9.1 Patterns by location

Across all locations, most sequences unsurprisingly belonged to Basidiomycota and Ascomycota. These dominant phylum trends somewhat follow the patterns of global soil fungal distribution, which Tedersoo et al (2014) reported as follows: Basidiomycota (55.7%), Ascomycota (31.3%), Mortierellomycotina (6.3%), and Mucoromycotina (4.4%), with the exception that in all study areas here, and both sampling methods, Ascomycota was represented by a higher proportion of OTU's than Basidiomycota.

9.2 Patterns by sampling type

As statistical analyses could not be applied to quantitatively assess differences between air and soil samples, observations were made from the KRONA charts. There were already noticeable differences at the level of phylum. In three of the five locations, soil samples had a relatively large proportion of OTU's classified to Zygomycota. In all cases this group was primarily composed of Mucorales and Mortierellales, which more recently have been recognized as their own phyla. Tampere had the largest proportion of Zygomycota, comprising 19% of all sampled soil fungi, followed by Joensuu at 10% and Jyväskylä at 4%. Helsinki and Lahti overall soil fungi profiles were less than <1% Zygomycota. Despite variation between locations, the presence of this group of fungi was a clear difference between soil and air, where it was always negligible. The difference is likely explained by lifestyle and distribution characteristics, since Mucorales and Mortierelles are mostly saprotrophs of soil detritus. Some Zygomycota species contain their spores in droplets of water and do not rely on air dispersal, but rather small animals (Malloch, 2020), which are more reliable in their habitat.

More distinctions between soil and air were found within phylum. Of Basidiomycota, both air and soil are, in every case, majority agaricomycetes. This aligns with global surveys of fungi, in which agaricomycetes are proportionally the largest major taxonomic group across all biomes (Tedersoo et al., 2014). However, the composition of agaricomycetes is where I found repeating patterns of major discrepancy between air and soil, and still further major differences among locations.

Soil agaricomycetes were composed of between 10% (Joensuu) and 37% (Tampere) Atheliales, which was further broken down to mostly ectomycorrhizal lineages like Piloderma, Tylospora, and Anphinema fungi. While some of these fungi produce spores, it's unsurprising that they were well-represented in soil profiles, where they spend most of their life, and barely present in the air samples. This result aligns with a survey of corticioid fungi in North America that also found that soil samples were predominately ectomycorrhizal (Rosenthal et al., 2017). On the other hand, agaricomycetes in air samples were always majority Polyporales (ranging from 36-52% of agaricomycetes), an order which never reached one percent of fungi in soil samples. Again, this trend is intuitive, from the lifestyle of polypores, which typically grow on wood and produce large spore-releasing fruit bodies.

10. Quantitative analyses

Fungal soil communities are more similar among locations (R=0.11, p=0.001) than among habitat types within a location. Air samples have even greater similarity of relative abundance among locations (R=0.08, p=0.001). The biggest differences are seen in between individual plots for soil (R=0.57, p=0.001), but not so much in air (R=0.18, p=0.001). In soil, community composition is fairly different between samples from different habitat types (R=0.38, p=0.001), but whether the sample is taken from the core or edge of the habitat does not make a bigger impact (R=0.24, p=0.001). In air samples, the difference in relative OTU abundance based on habitat is much lower (R=0.08, p=0.001) than soil.

Discussion

The primary hypothesis (H1) at the start was that like with many other studied organisms, community composition would change in relation to the physical distance between community sampling points, which was not well demonstrated by this study. While locations reached distances of over 300 km apart, the relative abundance of OTU's within these communities was not considered different based on the analysis of similarity. Most likely this result is due to the high dependence on landscape characteristics, which in terms of land-use patches, elevation, dominant vegetation, etc., are relatively homogenous in southern Finland.

Statistical analyses could not address differences in functional groups, because of the limitations of working with datasets containing only unspecified OTU's, therefore the hypothesis that functional groups would be differently affected by distance and habitat characteristics (H2) could not be quantified. However, qualitative analysis of KRONA charts did allow observations to be made indicating that fungal functional groups differ, substantially between sample type, and slightly between location. KRONA charts had urban and natural effects compiled within a site, making comparison of functional group between habitat type impossible. It has been previously found that climate, soil, and vegetation factors are strong predictors of the composition of functional groups (Tedersoo et al., 2014), and richness of ectomycorrhizal versus saprotrophic groups are related to soil acidity and plant host richness (Wardel & Lindahl, 2014) it would be valuable to use similar methods but separate habitat types in the qualitative analysis tools, so see if the different factors in urban settings strongly impact functional composition.

Analysis of similarity tests on relative abundance showed that soil samples were more different for all variables than air samples, providing evidence in support of the hypothesis that rates of distance-decay would be higher in soil samples than in air samples (H3).

As expected, the communities were different between urban and natural sites. While I hypothesized that community change would follow an urban to rural gradient (H4), this was not the case. Where differences were found, it was not gradual with urbanization, but rather a change immediately between urban, including edge and natural, including edge. As the differences between habitat type was more pronounced in soil types, the reason probably has less to do with the physical barriers to spore dispersal, and more about the differences in soil habitat characteristics. This is likely due to more variability in urban plots, than in forest plots which often had large, unbroken patches of homogenous forest cover.

In addition to the overall increased variation, urban soil has different properties in terms of pollutants, likely leading to further differences in which fungal species thrive. In the urban area of Helsinki, pollution leads to higher quantities of SO_2 and NO_x and certain heavy metals, which researchers found to be associated with lower soil respiration rates and less total fungal hyphae (Fritze 1988).These pollution effects dissipate away from city center and high traffic areas rapidly enough to be noticeable on the scale of this study's within-location sampling area; Viikki (Helsinki urban edge) has nearly double, and Helsinki core sites have three to four times higher NO_x concentrations compared to Nuuksio area (Helsinki natural sites) (Manninen et al., 2013). Inventories of fungi based on fruit body and sporocarp collection has previously shown an increase of saprotrophs and a decrease of ectomycorrhizal species towards emission sources, along a pollution gradient near Oulu, Finland (Tarvainen et al., 2003). Pollution from industry and travel could be one reason why fungal communities were different from natural at each urban site. What is surprising is that this trend was noticed across all urban areas, despite the differences in urban patch size and population density.

Certain statistical analyses were limited, both by the data format in some cases where there was only OTU's and no species information, and by the complexity necessary to calculate exact rates of change by precise distance. These would be areas for further studies to address more in-depth for future endeavors.

While these results demonstrate some of the current differences in fungal communities across various scales, the causal mechanisms for dispersal need more study. To disentangle underlying key drivers of ecological processes, contributing environmental conditions must be individually examined (Kivinen, 2007)

Because of the large effects observed from urbanization, insight may be gained by taking measurements of the microclimate in the same plots where fungal samples are collected. With these results, we know that the urban environment has an impact on fungi, but the precise mechanisms can only be guessed, using the still small body of information on what conditions impact fungal community establishment and structure. Therefore, more specific effects within the urban environment could be analyzed with additional data on atmospheric and soil conditions, including temperature and humidity, chemical composition, pollution, etc.

Similar studies, accounting for the broad fungal kingdom, should be conducted over longer periods of time and throughout all parts of the year. This study is only a snapshot of fungal community structure. Temporal effects can have a major impact on mushroom phenology, so from year to year there may be significant differences in the time when fungi fruits and releases spores (Pinna et al., 2010). Soil fungal communities in boreal forests also demonstrate temporal shifts, with saprotrophs dominating during the winter, and mycorrhizal dominating during the plants' growing season (Santalahti et al., 2016). Capturing many snapshots throughout the year could possibly show more difference in both spore samples and soil samples throughout the seasons.

Finally, similar types of studies should be conducted on fungal communities in more regions and climates. The region of Finland in this study is relatively homogenous in terms of habitat type and landscape mosaic. While this was useful for the purpose of minimizing certain external factors and maximizing likely effects from individual and dispersal characteristics, similar studies carried out in landscapes with more steep habitat gradients or dispersal barriers will contribute other patterns to help create a more complete picture of fungal community distance decay, from which more conclusions about contributing factors can be drawn. Since most Finnish cities, including three of the five used for this study (Lahti, Joensuu, and Jyvaskala) do not satisfy the criteria of urban used more broadly, such as the definition from the European Commision, which classifies urban clusters as having 300 inhabitants or more, per square

kilometer (Dijkstra & Poelman, 2014). As the strongest pattern of community change was between natural and urban habitats, even in these areas where the urban area is relatively small with low populations, effects of urbanization may be less pronounced here than other studies which consider denser and more impacted urban areas.

Acknowledgements

This thesis was made possible by the support of my advisor, Dr. Otso Ovaskainen, who provided guidance in many aspects, as well as the Research Centre for Ecological Change as a whole. Guidance was also provided by Dr. Nerea Abrego, who is using the data I collected in her own analyses and research paper, of which I am a co-author, and which is simultaneously being submitted for publication.

The cyclone samplers used to collect spores were generously loaned by the Global Spore Sampling Project.

Feild work was conducted with the help of collaborators from each of the different sampling locations: Jenna Purhonen, with the University of Jyväskylä, Karoliina Hämäläinen and Kaisa Junninen with the University of Eastern Finland in Joensuu, Minna Maunula with the University of Helsinki, and Amir Abdi in Tampere.

Clustering of DNA results and the creation of OTU tables was done by Panu Somervuo with the Organismal and Evolutionary Biology Research Programme at the University of Helsinki.

References

Abarenkov, K., Somervuo, P., Nilsson, R. H., Kirk, P. M., Huotari, T., Abrego, N., & Ovaskainen, O. (2018). Protax-fungi: a web-based tool for probabilistic taxonomic placement of fungal internal transcribed spacer sequences. *New Phytologist*, *220*(2), 517-525.

Abrego, N., Garcia-Baquero, G., Halme, P., Ovaskainen, O., & Salcedo, I. (2014). Community turnover of wood-inhabiting fungi across hierarchical spatial scales. PLoS ONE, *9*(7) doi: 10.1371/journal.pone.0103416

Abrego, N., Norros, V., Halme, P., Somervuo, P., Ali-Kovero, H. and Ovaskainen, O. (2018). Give me a sample of air and I will tell which species are found from your region – molecular identification of fungi from airborne spore samples. *Molecular Ecology Resources, 00:* 1–14. https://doi.org/10.1111/1755-0998.12755

Alberti, M., & Marzluff, J. M. (2004). Ecological resilience in urban ecosystems: linking urban patterns to human and ecological functions. *Urban ecosystems*, *7*(3), 241-265.

Bahram, M., Põlme, S., Kõljalg, U., Zarre, S., & Tedersoo, L. (2012). Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist*, *193*(2), 465-473.

Bahram, M., Koljalg, U., Courty, P. E., Diedhiou, A. G., Kjøller, R., Polme, S., Ryberg, M., Veldre, V. & Tedersoo, L. (2013). The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. *Journal of Ecology*, *101*(5), 1335-1344.

Bahram, M., Peay, K. G., & Tedersoo, L. (2014). Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. *New Phytologist, 205*(4), 1454-1463. doi:10.1111/nph.13206

Bonkowski, M., Griffiths, B. S., & Ritz, K. (2000). Food preferences of earthworms for soil fungi. *Pedobiologia*, *44*(6), 666-676. doi:10.1078/s0031-4056(04)70080-3

Carreiro M. M., K. Howe, D. F. Parkhurst, and R. V. Pouyat. (1999). Variations in quality and decomposability of red oak litter along an urban-rural land use gradient. *Biology and Fertility of Soils* 30: 258-68.

Chust, G., Pérez-Haase, A., Chave, J., & Pretus, J. L. (2006). Floristic patterns and plant traits of Mediterranean communities in fragmented habitats. *Journal of Biogeography*, *33*(7), 1235-1245.

Dale, M. R., & Fortin, M. J. (2014). *Spatial analysis: a guide for ecologists*. Cambridge University Press.

Dijkstra, L., & Poelman, H. (2014). *A harmonised definition of cities and rural areas: The new degree of urbanisation* (WP 01/2014) (European Commision)

Fritze, H. (1988) Influence of urban air pollution on needle litter decomposition and nutrient release, *Scandinavian Journal of Forest Research, 3*(1-4), 291-297, DOI: 10.1080/02827588809382517

Garcillán, P. P. & Ezcurra, E. (2003) Biogeographic regions and ß-diversity of woody dryland legumes in the Baja California peninsula. *Journal of Vegetation. Science 14*: 859–868.

Gómez-Hernández, M., Williams-Linera, G., Guevara, R., & Lodge, D. J. (2012). Patterns of macromycete community assemblage along an elevation gradient: options for fungal gradient and metacommunity analyse. *Biodiversity and Conservation*, *21*(9), 2247-2268.

Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M. *et al.* (2004). Spatial scaling of microbial eukaryote diversity. *Nature*, 432, 747–750.

Häkkinen, M., Heikkinen, J., & Mäkipää, R. (2011). Soil carbon stock increases in the organic layer of boreal middle-aged stands. *Biogeosciences, 8*(5), 1279-1289. doi:10.5194/bg-8-1279-2011

Ivanova NV, Fazekas AJ, Hebert PDN. (2008). Semi-automated, membrane-based protocol for DNA isolation from plants. Plant Molecular Biology Report, 26(3), 186–198.

Jauker, F., Diekoetter, T., Schwarzbach, F., & Wolters, V. (2009). Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. *Landscape Ecology*,*24*(4), 547-555.

Jumpponen, A., Jones, K. L., & Blair, J. (2010). Vertical distribution of fungal communities in tallgrass prairie soil. *Mycologia*,*102*(5), 1027-1041.

Kranabetter, J. M., Durall, D. M., & MacKenzie, W. H. (2009). Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza*, *19*(2), 99-111.

Kornis, M. S., Weidel, B. C., Powers, S. M., Diebel, M. W., Cline, T. J., Fox, J. M., & Kitchell, J. F. (2015). Fish community dynamics following dam removal in a fragmented agricultural stream. *Aquatic Sciences, 77*(3), 465-480.,

Kowarik I., H. Sukopp, S. Mejny (1990). Some responses of flora and vegetation to urbanization in Central Europe. *Urban ecology: Plants and plant communities in urban environments* 45-74.

Malloch, D., (2020). *Zygomycota*. [online] Website.nbm-mnb.ca. Available at: <http://website.nbm-mnb.ca/mycologywebpages/NaturalHistoryOfFungi/Zygomycota.html> [Accessed 1 April 2020].

Manninen, S., Sassi, M. K., & Lovén, K. (2013). Effects of nitrogen oxides on ground vegetation, Pleurozium schreberi and the soil beneath it in urban forests. *Ecological indicators*, 24, 485-493.

McDonnell, M.J., Pickett, S.T., Groffman, P., Bohlen, P., Pouyat, R.V., Zipperer, W.C., Parmelee, R.W., Carreiro, M.M. & Medley, K. (2008). Ecosystem processes along an urban-to-rural gradient. In Urban Ecology (pp. 299-313). Springer, Boston, MA.

Meentemeyer, V., & Box, E. O. (1987). Scale effects in landscape studies. In *Landscape heterogeneity and disturbance* (pp. 15-34). Springer, New York, NY.

Nekola, J. C., & White, P. S. (1999). The distance decay of similarity in biogeography and ecology. *Journal of Biogeography*,*26*(4), 867-878.

Niemelä, J. (1999) Ecology and urban planning. *Biodiversity and Conservation,* 8: 119.https://doi.org/10.1023/A:1008817325994

Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A.I., Basset, Y., Janda, M. *et al.* (2007). Low beta diversity of herbivorous insects in tropical forests. *Nature*, *448*, 692–697.

Official Statistics of Finland (OSF): Population structure [e-publication]. ISSN=1797-5395. Helsinki: Statistics Finland [referred: 12.10.2019]. Access method: http://www.stat.fi/til/vaerak/index_en.html

Oke, T. R. (1973). City size and the urban heat island. *Atmospheric Environment, 7*(8), 769-779.

Ondov, B. D., Bergman, N. H., & Phillippy, A. M. (2011). Interactive metagenomic visualization in a web browser. *BMC Bioinformatics*, *12*(1), 385

Ovaskainen O., Abrego N., Somervuo P., Palorinne I., Hardwick B., Pitkänen J.M., Andrew N.R., Niklaus P.A., Schmidt N.M., Seibold S., Vogt J., Zakharov E.V., Hebert P.D.N., Roslin T. & Ivanova N.V. (2020) Monitoring Fungal Communities With the Global Spore Sampling Project. *Frontiers in Ecology and Evolution. 7*:511. doi: 10.3389/fevo.2019.00511

Pavao-Zuckerman, M. A., & Coleman, D. C. (2007). Urbanization alters the functional composition, but not taxonomic diversity, of the soil nematode community, *Applied Soil Ecology*, *35*(2), 329-339. doi:10.1016/j.apsoil.2006.07.008

Peay, K. G., Bidartondo, M. I., & Elizabeth Arnold, A. (2010). Not every fungus is everywhere: scaling to the biogeography of fungal–plant interactions across roots, shoots and ecosystems. *New Phytologist*, *185*(4), 878-882.

Peay, K. G., & Bruns, T. D. (2014). Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant–fungal interactions. *New Phytologist*, *204*(1), 180-191.

Pérez-del-Olmo, A., Fernández, M., Raga, J. A., Kostadinova, A., & Morand, S. (2009). Not everything is everywhere: the distance decay of similarity in a marine host–parasite system. *Journal of Biogeography*, *36*(2), 200-209.

Pickett, S. T., & Cadenasso, M. L. (1995). Landscape ecology: spatial heterogeneity in ecological systems. *Science*, *269*(5222), 331-334.

Pinna, S., Gévry, M. F., Côté, M., & Sirois, L. (2010). Factors influencing fructification phenology of edible mushrooms in a boreal mixed forest of Eastern Canada. *Forest ecology and management*, *260*(3), 294-301.

Qian, H. & Ricklefs, R.E. (2007). A latitudinal gradient in large-scale beta diversity for vascular plants in North America. *Ecology Letters*, *10*, 737–744.

Qian, H., Badgley, C. & Fox, D.L., (2009). The latitudinal gradient of beta diversity in relation to climate and topography for mammals in North America. *Global Ecology and Biogeography*, *18*(1), 111-122.

Rapport, E. H. (1993). The process of plant colonization in small settlements and large cities. In Humans as components of ecosystems, ed. M. J. McDonnell and S. T. A. Pickett, 190-207. New York:Springer-Verlag)

Rosenthal, Lisa M., Karl-Henrik Larsson, Sara Branco, Judy A. Chung, Sydney I. Glassman, Hui-Ling Liao, Kabir G. Peay, Smith, D.P., Talbot, J.M., Taylor, J.W. & Vellinga, E.C. (2017). Survey of corticioid fungi in North American pinaceous forests reveals hyperdiversity, underpopulated sequence databases, and species that are potentially ectomycorrhizal. *Mycologia 109*(1), 115-127.

Rudnicky, J. L., & McDonnell, M. J. (1989). Forty-eight years of canopy change in a hardwood-hemlock forest in New York City. Bulletin of the Torrey Botanical Club 116: 52-64

Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T., & Heinonsalo, J. (2016). Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. *FEMS microbiology ecology*, *92*(11).

Soininen, J., McDonald, R., & Hillebrand, H. (2007). The distance decay of similarity in ecological communities. *Ecography*,*30*(1), 3-12.

Somervuo, P., Koskela, S., Pennanen, J., Nilsson, R. H., & Ovaskainen, O. (2016). Unbiased probabilistic taxonomic classification for DNA barcoding. *Bioinformatics*, *32*(9), 2920–2927.

Steinberg, D. A., Pouyat, R. V., Parmelee, R. W., & Groffman, P. M. (1997). Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient. *Soil Biology and Biochemistry*, *29*(3-4), 427-430.

Taha, H. (1997). Urban climates and heat islands: albedo, evapotranspiration, and anthropogenic heat. *Energy and buildings*,*25*(2), 99-103.

Tarvainen, O., Markkola, A. M., & Strömmer, R. (2003). Diversity of macrofungi and plants in Scots pine forests along an urban pollution gradient. *Basic and Applied Ecology*, 4(6), 547-556.

Taylor, P. H., & Gaines, S. D. (1999). Can Rapoports Rule Be Rescued? Modeling Causes of the Latitudinal Gradient in Species Richness. *Ecology*, *80*(8), 2474. doi:10.2307/177233

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A. & Smith, M.E. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), p.1256688.

Vernes, K., & Dunn, L. (2009). Mammal mycophagy and fungal spore dispersal across a steep environmental gradient in eastern Australia. *Austral Ecology*, *34*(1), 69-76.

Wardel, D., & Lindahl, B. (2014). Disentangling global soil fungal diversity. Science, 346(6213), 1052–1052. doi: 10.1126/science.aaa1185

Wernick, B. G., K. E. Cook, & H. Schreier. (1998). Land use and streamwater nitrate-N dynamics in an urban-rural fringe watershed. *Journal of the American Water Resources Association* 34: 639-50

Wiens, J. A. (1989). Spatial scaling in ecology. *Functional ecology*, *3*(4), 385-397

Wiens, J. A., Chr, N., Van Horne, B., & Ims, R. A. (1993). Ecological mechanisms and landscape ecology. *Oikos*, *66*: 369-380.

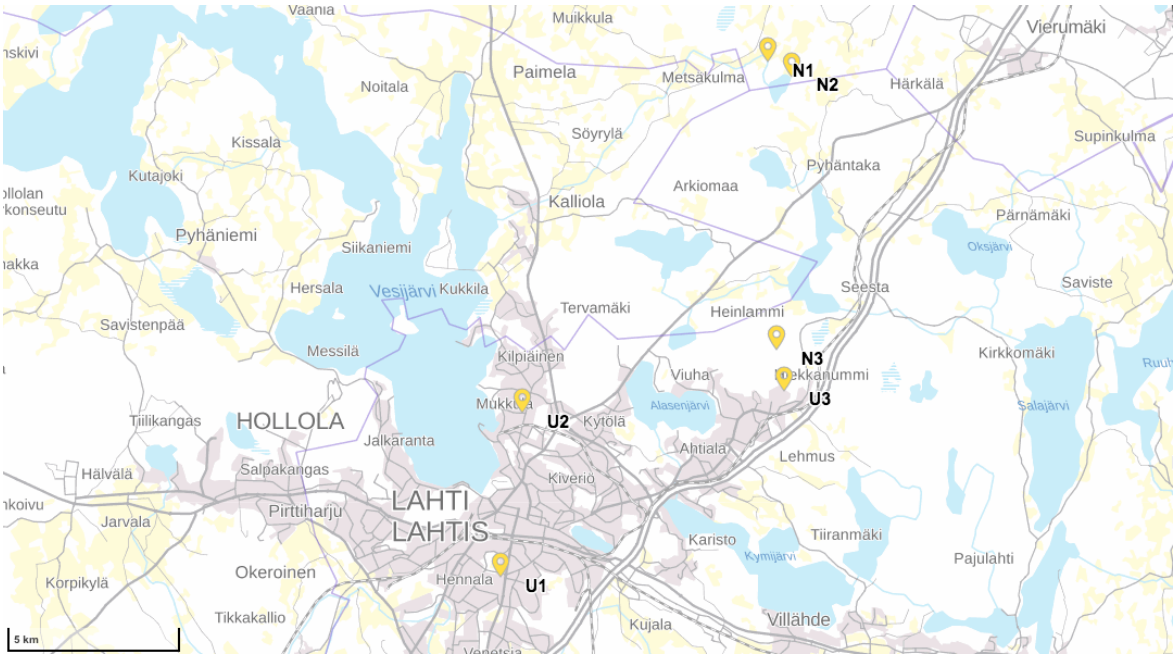
Willems, J. J. G. M., Marinissen, J. C. Y., & Blair, J. (1996). Effects of earthworms on nitrogen mineralization. *Biology and fertility of soils*, *23*(1), 57-63.

World Population Review (2019), Population of Cities in Finland (2019). Retrieved August 15, 2019, fromhttp://worldpopulationreview.com/countries/finland-population/cities/

Xiao, D., & Li, X. (1997). Spatial ecology and landscape heterogeneity. *Acta Ecologica Sinica*, *17*(5), 543-461.

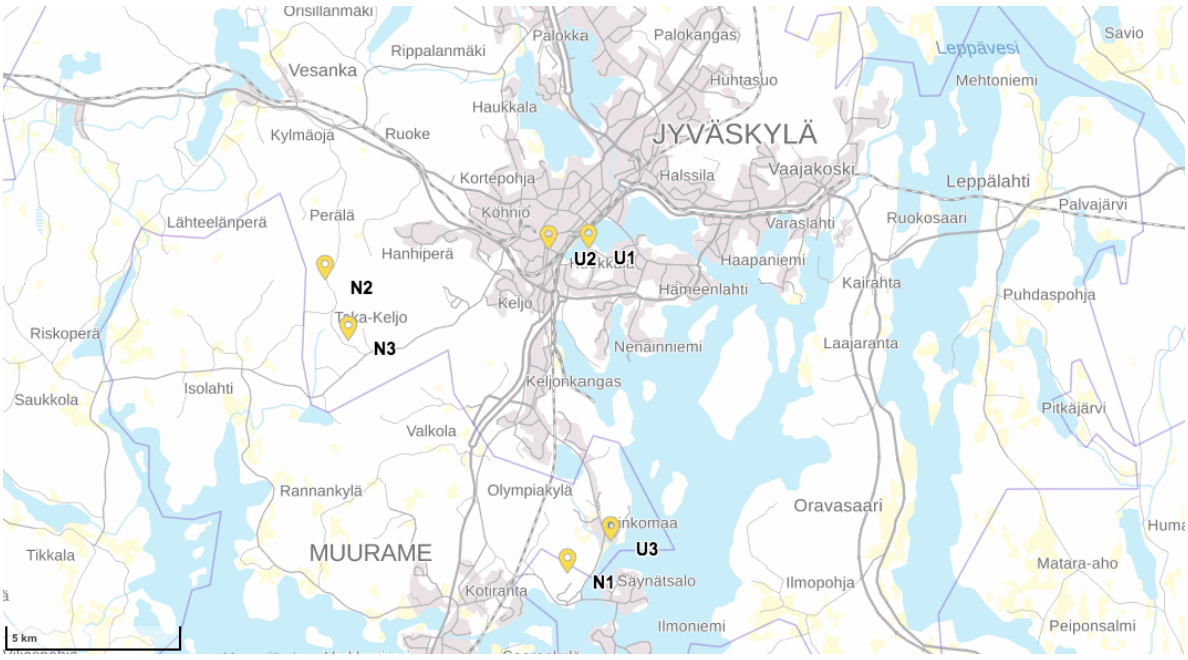
Helsinki

Lahti



Tampere

Jyväskylä



Joensuu


Hirviranta
Kaiskunvaara
Sarvinki
Ahokkala
Mönni
Saavansalo
Kangasvesi
Suoniemi
Paihola
Puntarikoski
Kylmäoja
Kaisakanlahti
Lehmo
Sipri
Elovaara
Selkie
Kotalahti
Jukajoki
Onttola
Marjosärkkä
Jokiranta
Lehtoi
Kansanaho
Utramo
Raatekangas
Kuplu
U3
N3
N3
Marjala
Mustavaara
N2
N1
Utra
Kulho
Mutala
Aavaranta
Multimäki
U2
Otsola
U1
Karsikko
Alavi
Röksä
Ylinen
JOENSUU
Iiksenvaara
Keskijärvi
Hukanhauta
Iiksenniitty
Pohjoispää
Vehkaniemi
Lahdenperä
Karhunmäki
Heinävaara
Ketunpesät
Särkivaara
anta
5 km

Appendix B

Excerpt from Ivanova (2008) DNA extraction protocol:

Air samples were processed as described in our GSSP manuscript. Soil samples were extracted as follows:

One 3/8' sterile stainless steel bead and 3-5 ml of ILB+1%PVPP buffer with 25 µl of Proteinase K (20 mg/ml) per each 1 ml of buffer were added to each sample. Soil samples were homogenized on Genie 2 vortex on max speed for 5 min. Tubes were incubated at 56°C for 2 hours on the orbital shaker, followed by 2 hours incubation at 65°C. Tubes were centrifuged for 5 min at 2000 xg and a volume of 200 µl of each lysate was transferred to a 500 µl Eppendorf deep-well plate using Biomek NX; 100 µl of lysate from each replicate was mixed with 200 µl of 5M GuSCN Plant Binding buffer and applied to a 96-well Acroprep plate with 1 µm Glass Fiber membrane (PALL) for DNA binding. The washes were conducted at 5000xg as described in [1] with the following modifications: 1st wash – 300 µl of 5M GuSCNbuffer; 2nd wash – 300 µl of plant PWB; 3rd and 4th washes – 600 µl of WB. After the last wash plate was incubated at 56°C to dry the membrane; DNA was eluted in 80 µl of 10 mM Tris-HCL pH 8.0.

Because some soil samples contained too much soil in the tube, DNA was not completely purified from humic acids, resulting in dark brown DNA extracts.

Therefore 70 µl of each DNA extract was transferred to a 500 µl Eppendorf deep-well plate containing 70 µl of 1% ILB + PVPP and 280 µl of 5M GuSCN binding buffer; 1st wash - 150 µl of 5M GuSCN binding buffer, followed by two washes with 600 µl of WB. After the last wash plate was incubated at 56°C to dry the membrane; DNA was eluted in 70 µl of 10 mM Tris-HCL pH 8.0.